18 Applications of Immunomagnetic Beads and Biosensors for Pathogen Detection in Produce

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Introduction

Recent outbreaks of pathogenic bacteria on produce have been widespread with severe consequences. Consuming hepatitis A–contaminated green onions sickened 555 persons and killed 3 from a restaurant in western Pennsylvania (CDC 2003). In October 2006, spinach contaminated with *E. coli* O157:H7 infected 199 people in 28 states, resulting in 141 hospitalizations, 31 cases of kidney failure, and 3 deaths (Surak 2007). Shortly thereafter, lettuce contaminated with *E. coli* O157:H7 associated with Taco Bell had 71 reported cases, with 53 reported hospitalizations and 8 cases of kidney failure (CDC 2006). Particularly susceptible to foodborne illness are the very young, the elderly, individuals with existing diseases, or immunocompromised individuals. In addition to the human toll, these outbreaks of foodborne illness have caused severe economic losses to food companies, consumers, and employers in general.

Escherichia coli 0157:H7 has been implicated with increasing frequency from outbreaks associated with fresh produce, including bean sprouts, cantaloupes, apples, lettuce, spinach, tomatoes, etc. (Ackers and others 1998; Hillborn and others 1999). The mechanisms by which the pathogen is introduced are not fully understood; however, one hypothesis states that the plants may have been contaminated in fields by exposure to contaminated animal feces and/or improperly treated manure (Beuchat 1999). Current epidemiological data indicate that *E. coli* 0157:H7 may be present in up to 8.3% of dairy and beef cattle (Faith and others 1996) and that it is shed asymptomatically in the feces. Although current manure-handling guidelines recommend a composting period to reduce microbial pathogens in manure before its application as a field fertilizer (FDA 1998), research has demonstrated that manure can support the long-term survival of *E. coli* 0157:H7 in a variety of conditions (Kudva and others 1998; Wang and others 1996). A second vehicle by which *E. coli* 0157:H7 may be introduced is flood irrigation with water contaminated with cattle feces or contact with contaminated surface runoff (Ackers and others 1998; Hillborn and others 1999). A number of recent *E. coli* 0157:H7 outbreaks have been linked to contaminated water (CDC 1999); furthermore, studies have demonstrated the ability of the pathogen to survive for extended periods in water (Chalmers and others 2000; Wang and Doyle 1998).

The dose for *E. coli* 0157:H7 to cause human illness is very low and may be as few as 10 organisms (FDA 1998). Some victims, particularly the very young, have developed the hemolytic uremic syndrome (HUS), which is characterized by renal

failure and hemolytic anemia. From 0 to 15% of hemorrhagic colitis victims may develop HUS, which can lead to the permanent loss of kidney function. In the elderly, HUS, plus two other symptoms, fever and neurological symptoms, constitutes thrombotic thrombocytopenic purpura (TTP). The mortality rate of this condition in the elderly can be as high as 50%. Thus, there is a need of rapid and sensitive methods to detect this pathogen in foods.

Traditional bacterial pathogen detection methods can take several days to confirm a positive sample. In the case of *Campylobacter*, culturing and plating takes 14–16 days for a positive result (Brooks and others 2004). Different selective media are used for the detection of particular bacteria species. They can contain inhibitors (in order to stop or delay the growth of nontargeted strains) or particular substrates that only the targeted bacteria can degrade or that confers a particular color to the growing colonies, such as rainbow agar for *Salmonella* detection. (Fratamico 2003). Although these methods are inexpensive they are very time consuming. To assure the safety of our foods and a rapid response to help those afflicted, clearly more rapid detection methods are needed.

Biosensors for Pathogen Detection

Efforts to develop alternative food pathogen detection methods in general include the development of biosensor technologies for pathogen detection. Biosensors use biological receptor compounds (e.g., antibody, enzyme, nucleic acid, etc.) and the transduction of the molecular interaction through changes in physical and/or physicochemical properties in real time to detect the presence of the entity specific to the bioreceptor (Leonard and others 2003) Principally, there are four types of biosensors that measure signal transduction through changes in optical, mass, electrochemical, and thermal properties (Goepel 1991; Seyhi 1994; Goepel and Heiduschka 1995). Some of the general principles and applications of biosensors are briefly described as follows.

Optical biosensors based on the evanescent wave (EW) use the technique of attenuated total reflection (ATR) spectroscopy and surface plasmon resonance (SPR) to measure real-time interaction between biomolecules. The basis of ATR is the reflection of light inside the core of a waveguide when the angle of incidence is greater than the critical angle. Waveguides can be slab guides, planar integrated optics, or optical fibers. Light waves are propagated along waveguides by the law of total internal reflection (TIR). Even though the light is totally internally reflected, the intensity does not abruptly fall to zero at the interface, resulting in generation of evanescent wave (EW), which penetrates exponentially into the medium of lower refractive index (Squillante 1998). The wavelength of light, ratio of the refractive indices, and angle of the light at the interface determine the penetration depth (Anderson and others 1993), which are typically 50 to 1000 nm; thus the EW is able to interact with many monolayers at the surface of waveguides (Lave and others 1991). Reactions occurring very close to the interface perturb the evanescent wave, and the changes in signals can be related to the amount of binding between the target and immobilized ligand at the interface.

When metal surfaces are used to immobilize the receptors, the change in EW induced by the surface binding may also change the plasmon resonance of the surface

metal layer (SPR) (Tubb and others 1997). Thus, the main difference between ATR and SPR is that the former measures the changes of EW at the interphase directly and the latter measures the induced changes in the resonant excitation of the free electrons of the metal layer providing the anchoring sites for the specific receptor. Both ATR (Geng and others 2006) and SPR (Fratamico 1998) have been applied to measure food pathogens.

Acoustic wave biosensors are based on the decrease of oscillating frequency of bioreceptor-coated piezoelectric crystals upon the binding of target analyte. The change in frequency is governed by the ratio of the mass of analyte and the piezoelectric crystal (Griffiths and Hall 1993). Recently, this type of approach has been applied to measure *E.* coli O157:H7 (Campbell and Mutharasan 2007). The sensitivity of this type of sensor is superior. However, the fabrication and treatment of the crystal require considerable technical training and expertise (Invitski and others 1999).

The electrochemical sensor involves the use of a receptor-coated electrode that expresses a change in electro-properties upon the binding of target analyte. The best known electrochemical devise for measuring specific analyte is the glass pH-electrode that expresses potential change upon the binding of protons on the glass surface. In the medical field, the most widely used electrochemical sensor is the glucose monitoring sensor (D'Costa and others 1986). This approach has also been applied to measure food pathogens using alkaline phosphatase labeled antibodies to link to the bacteria that were captured by the antibodies immobilized on the electrode (Gehring and others 1996). The enzyme then was used to convert phenolic phosphate to phenolic compound that could be characterized by its specific redox potential. The magnitude of the redox current could relate to the number of pathogen captured.

Thermometric biosensors exploit the fundamental property of biological reactions, i.e. absorption or evolution of heat (Spink and Wadsö 1976). This is reflected as a change in the temperature within the reaction medium. Its exploitation in biosensors led to the development of thermometric devices (Mosbach and Danielsson 1974). These predominantly measure the changes in temperature of the circulating fluid following the reaction of a suitable substrate with the immobilized enzyme molecules. The most basic version of such a device is a thermometer, routinely used for measurement of body or ambient temperature. Based on similar principles, in thermometric devices the heat is measured using sensitive thermistors. Such a device is popularly referred to as an enzyme thermistor, ET (Danielsson and Mosbach 1988). Several instruments were designed in the past 2 decades and they combined the principles of calorimetry, enzyme catalysis, immobilization on suitable matrices, and flow injection analysis for small metabolite detections. Because of its low sensitivity, the application of thermal sensor for pathogen detection has not yet been attempted.

The detection of microorganisms by DNA amplification has been extensively applied. Using polymerase chain reaction (PCR) target nucleic segments of defined length and sequence are amplified by repetitive cycles of strand denaturation, annealing, and extension of oligonucleotide primers by the thermostable DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase (Bsat and others 1994). PCR has distinct advantages over culturing and other methods for the detection of microbial pathogens and offers the advantages of specificity, sensitivity, rapidity, accuracy, and capacity to

detect small amounts of target nucleic acid in a sample (Toze 1999). PCR has been shown to accurately detect low numbers of microbes such as viruses (Schwab and others 1996) and bacteria (Jensen and others 1993; Fach and Popoff 1997). Multiple primers can be used to detect different pathogens from one multiplex reaction. However, this technique can be limited by problems such as the sensitivity of the polymerase enzyme to environmental contaminants, difficulties in quantification, generation of false positives through the detection of naked nucleic acids, nonviable microorganisms, or contamination of samples in the laboratory (Toze 1999). Nucleic acid-based assays can indicate only the genetic potential of a microorganism to produce toxin or to express virulence and do not provide any information on toxins in foods or environmental samples. From a practical point of view, the routine detection of microbes using PCR can be expensive and complicated, requiring highly skilled workers to carry out the tests.

All of these methods require samples of small volume and they may have the sensitivity to detect infectious dosage of pathogens in the sample (e.g., only a few cells of *E. coli* O157:H7). However, the low levels of pathogen may not be uniformly distributed, but highly localized in a food matrix. Assuring that the samples used for detection contain the target pathogen is a demanding challenge. To circumvent this problem, culture enrichment of multiple samples is often needed to increase cell concentration and thus enhance pathogen detection.

Alternatively, immunomagnetic beads (IMB) can be used to rapidly and effectively separate and concentrate targeted pathogens, and the method has attracted increased interest (Molday and others 1977; Sinclair 1998). IMB have been applied in several rapid methods to capture pathogens prior to analysis (Fratamico and others 1992; Olsvik and others 1994). IMB also have been used to increase the signal intensity by concentrating captured pathogens into smaller detection volumes (Gehring and others 1996; Yu and Bruno 1996). In the past, we developed detection processes that involved first capturing targeted pathogens in foods from briefly enriched cultures by the use of specific IMB (Tu and others 2001a). The captured pathogens were further conjugated with second antibodies labeled with signal generating tags. The sandwiched complexes involving IMB, targeted pathogens, and labeled antibodies could be processed by the use of suitable magnetic devices. The captured pathogens were then revealed by different optical and electrical approaches. With this general approach, we were able to detect ~1 CFU/g of target pathogens in meat samples in a standard 8-h shift (Tu and others 2001b). Some of those developed approaches were also applied to produce systems. In the following sections, we briefly summarize our experience of detecting pathogens in produce by IMB and biosensors.

Biosensor Processes Involving the Use of IMB for Pathogen Detection

For these approaches, micron-sized iron-containing beads coated with antibodies specific to antigens of target organisms form IMB that are used to capture those targets. The captured pathogens and excess IMB can be easily separated from other solution components and conveniently transferred to a desired detection environment by the use of high-strength neodymium boron iron magnets associated with an automated

and programmable instrument (e.g., KingFisher apparatus of ThermoFisher Scientific, Waltham, MA).

Several companies produce a variety of IMB, such as Dynal beads and Quantum Dots from Invitrogen Corp. (Carlsbad, CA), BioMag beads from Polysciences Inc. (Warrington, PA), ProMag beads from Bangs Laboratories (Fishers, IN), MACS Microbeads from Miltenyi Biotech Inc. (Auburn, CA), and MagSpheres from Luminex Corporation (Austin, TX). Our previous work has shown that based on hydrodynamic considerations, larger and denser beads have greater capturing efficiencies than lighter and smaller beads (Tu and others 2003a). These beads are used to capture bacteria or toxins from briefly enriched food samples, washed and then incubated with another antibody conjugated with a signaling tag that might generate easily detectable signals (e.g., absorption and fluorescence changes) through either enzymatic or chemical reactions (Tu and others 2001b; Li and others 2004).

Detection of Pathogens on Cantaloupe

Cantaloupes grow in contact with the earth, which increases their potential for contact with soilborne bacteria, fungi, insects, and animals. The possibility of a product becoming infected is compounded by contaminated irrigation water, improperly applied fertilizers, ineffective washing techniques, and poor hygiene practices of field workers. It has been shown that *E. coli* O157:H7 can survive up to 100 days in soil (Ingham and others 2004). Several outbreaks and recalls of cantaloupe have occurred, in particular a multistate outbreak strain of *Salmonella* on cantaloupes from Mexico that caused 133 cases from 2000–2002 (CDC 2002). In November 2006, more than 62,000 cases of cantaloupes from the western U.S. were recalled by Rio Vista, Ltd. of Rio Rico, Arizona, because routine sampling by the FDA tested positive for *Salmonella* (FDA 2006). Other microbes have been cited in outbreaks on cantaloupe, including *Campylobacter* and *Norovirus* (Bowen and others 2006). Because of its rough surface and porous veins, bacteria can attach to cantaloupe surfaces rather tightly, as evidenced by the difficulty in removing the bacteria through simple aqueous washings (Ukuku and others 2001). The bacteria may become incorporated into biofilms with existing microflora, which can further shield from the effects of washing or chemical treatments (Annous and others 2005). This noncompetitive relationship has been demonstrated by inoculating the surface with phytopathogenic mold, which does not inhibit the growth of subsequently inoculated *Salmonella* (Richards and Beuchat 2005). The waxy surface of the fruit can repel aqueous sanitizers (Beuchat and Ryu 1997). This strong attachment and hydrophobicity may add further complications to the detection, quantification, and reduction of suspected pathogens on the surfaces of cantaloupes. Sanitizing methods have included the use of hydrogen peroxide, chlorine, 94 °C water, (Ukuku 2006) as well as ozone, peroxyacetic acid, and chlorinated trisodium phosphate (Rogers and others 2004). Bacteria can be transferred to the flesh of the melons by cutting, which provides a surface that supports the growth of pathogenic bacteria (Del Rosario and Beuchat 1995). The possibility of cross-contamination from food service workers or other foods led to the proposal of a Hazard Analysis and Critical Control Point (HACCP) plan for handling of fresh produce (Beuchat 1995). Twenty cases of *E. coli* O157:H7 cross-contamination of cantaloupe were documented in Oregon in 1993 (Jackson and others 2000).

Because *E. coli* 0157:H7 bacteria can be a hazard on cantaloupe from the field as well as in postharvest handling, rapid and sensitive detection methods are needed. To determine the levels of *E. coli* 0157:H7 on cantaloupe, we tested the applicability of two different detection methods (Fig. 18.1) involving the use of immunomagnetic heads to first capture and concentrate *E. coli* 0157:H7 from cantaloupe samples. The captured bacteria (shown as B in the figure) were than detected either by the bioluminescence of cellular NAD(P)H or by a chemiluminescent sandwich assay. The results showed that the methods developed were capable of detecting relatively low levels of the *E. coli* 0157:H7 spiked on the surfaces of cantaloupes within 3.3 h (Tu and others 2004).

NAD(P)H Method

The NAD(P)H method mentioned above involves the measurement of cellular NAD(P)H via an externally added electron transfer system that uses membrane permeable menadione to oxidize internal NAD(P)H to $NAD(P)^+$. Menadione reduces molecular oxygen to hydrogen peroxide (H_2O_2) that generates chemiluminescent luminol by the action of horseradish peroxidase (Fig. 18.2). This reaction system utilized the cellular NAD(P)H and membrane-bound electron transfer process to produce luminol-supported and peroxidase-catalyzed chemiluminescence.

As described in our previous report (Tu and others 2004), the NAD(P)H method was useful to measure the presence of viable cells. Figure 18.2 shows the method

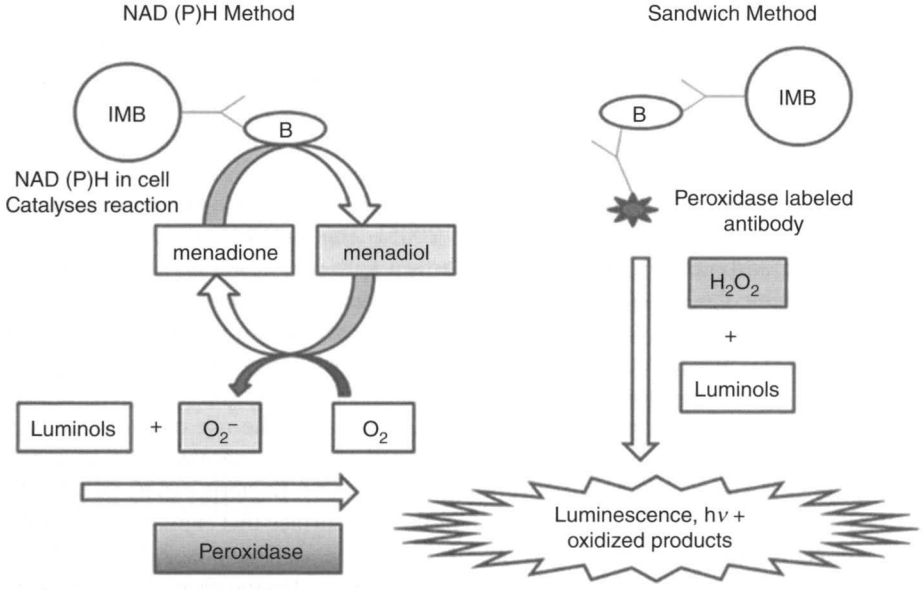

Figure 18.1. Two luminescent assays for bacterial detection. The bacteria (B) either provide the cellular reducing power in the forms of NAD(P)H or are sandwiched with peroxidase labeled antibody.

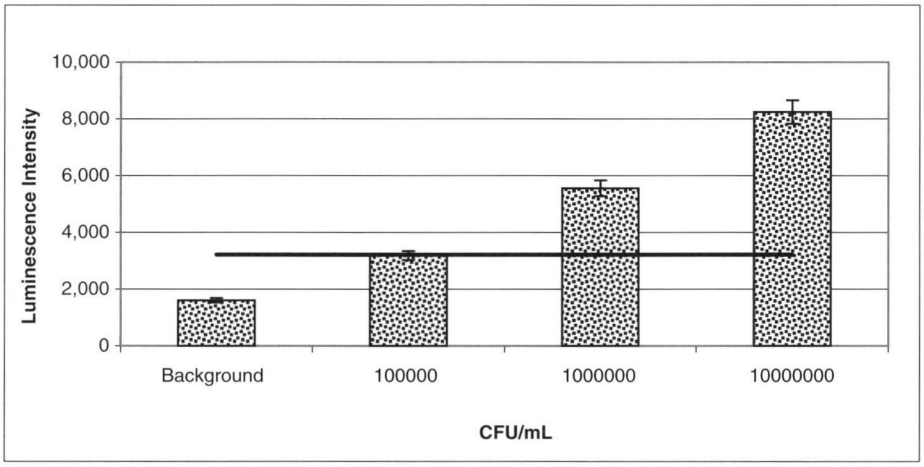

Figure 18.2. Sensitivity of NAD(P)H method.

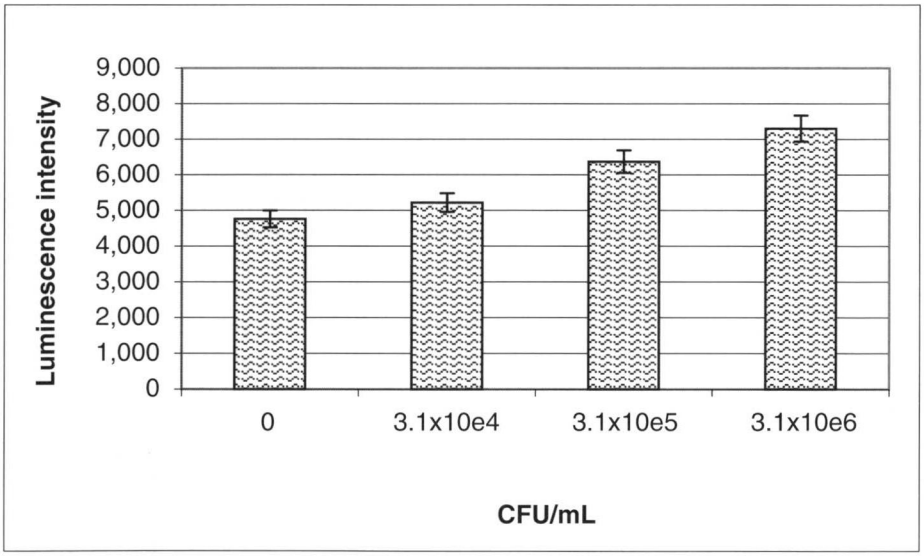

Figure 18.3. Detection levels of bacteria inoculated onto cantaloupe.

could measure 10^6 CFU/ml of *E. coli* O157:H7. The solid line in the figure represented twice the value of background and was arbitrarily chosen as the limit of detection.

We spiked cantaloupe surfaces with different levels of *E. coli* O157:H7 in circular areas of 2 cm diameter. After drying for 1 h to promote adhesion, the spiked area was removed as discs and incubated in nutrient broth for 3.3 h. As illustrated in Figure 18.3, the NAD(P)H method was able to detect the presence of ~3×10^6 CFU of the

bacteria in an area of ~3 cm^2 of cantaloupe surface. The background signal is higher in cantaloupe assay due to presence of other materials that increase the false positive (rind, pulp, endogenous bacteria). The detection after 3.3 h is noteworthy because the discs were added to 100 ml nutrient broth and only 200 µl were assayed.

Sandwich Method

This assay uses bacteria captured by beads and then incubated with peroxidase-labeled antibody against the target antigen. The beads are eluted into a luminol-based cocktail (BioFX Corp., Owings Mills, MD). The assay measures the activity of the peroxidase conjugated to anti–*E. coli* 0157 antibodies. The amount of activity is proportional to the concentration of bacteria captured by the beads. Unlike the NAD(P)H method, the sandwich method detects intact (viable and injured) and fragmented target cells, because the sandwich detection is relying on interactions between applied antibodies and antigens on bacterial surfaces. As shown in Figure 18.4, the sensitivity of the sandwich method in detecting cultured *E. coli* O157:H7 is more than 10 times better than that of the NAD(P)H method. The horizontal bar represents a value twice the background reading and is arbitrarily set as the limit of detection. Thus 100,000 CFU/ml is approximately the detection limit using this method.

This sandwich method was applied to detect *E. coli* O157:H7 spiked on cantaloupe as described for Figure 18.3. As shown in Figure 18.5, the sandwich method could detect ~1000 CFU/cm^2 of cantaloupe surface after an enrichment of 3.3 h (Tu and others 2004). As with the NAD(P)H method, the background of cantaloupe samples was higher than that of the pure cultures cells but not as relatively high as the NAD(P)H method. Thus, a lower number of cells can be detected after the incubation period using the sandwich method. The lower background and greater sensitivity make the sandwich test more desirable for detecting *E. coli* O157:H7 in cantaloupe samples.

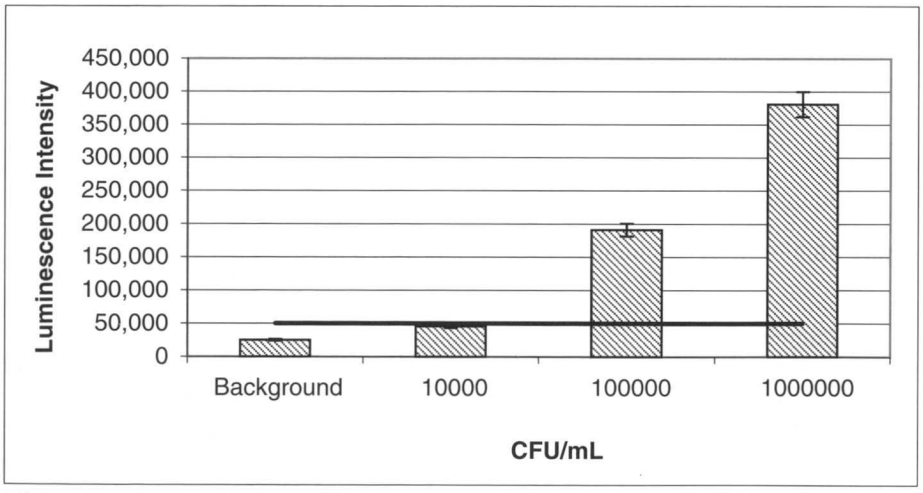

Figure 18.4. Sensitivity of the sandwich method.

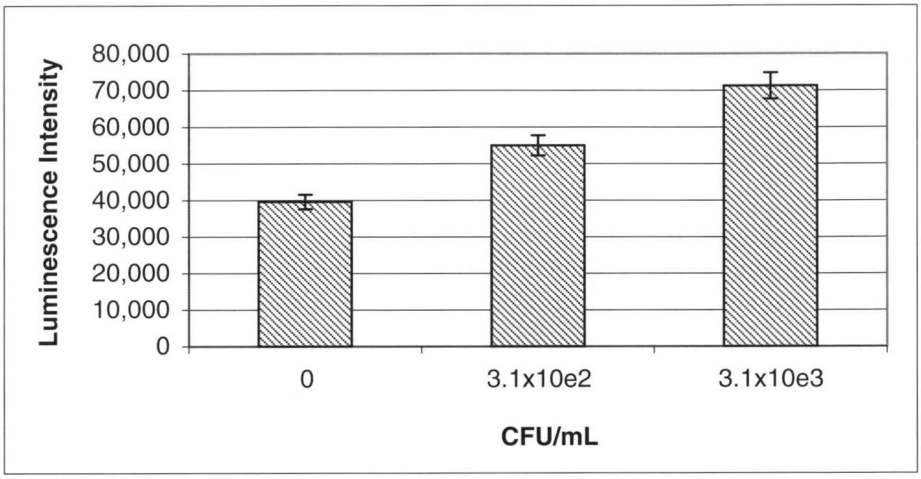

Figure 18.5. Sandwich method detection levels of bacteria inoculated onto cantaloupe.

Detection of Pathogens Associated with Alfalfa Sprouts

The use of uncooked sprouts as a salad ingredient has gained considerable acceptance by American consumers. However, outbreaks of *E. coli* 0157:H7 and *Salmonella* spp. associated with the consumption of raw sprouts have become a concern (Breuer and others 2001; Brooks and others 2001; CDC 2001; Ferguson and others 2005). Pathogenic bacteria on the seeds, if any, can rapidly grow to 10^6–10^8 CFU/g of product under the warm, moist, nutrient rich sprouting conditions (NACMCF 1999; Stewart and others 2001a). The internalization of the pathogenic bacteria into the edible parts of the sprouts, the cotyledons and hypocotyls, makes them difficult to disinfect after sprouting (Gandhi and others 2001; Hara-Kudo and others 1997; Ito and others 1998).

In 1999, the FDA recommended that sprout growers decontaminate seeds to reduce the microbial hazards of sprouts (FDA 1999). While currently approved treatments of seeds may reduce 99–99.9% of microbial populations, they do not guarantee a pathogen-free sprouted product (Beuchat and Ryu 1997; Brooks and others 2001; Jaquette and others 1996; NACMCF 1999; Pandrangi and others 2003; Proctor and others 2001; Stewart and others 2001b; Taormina and others 1999; Weissinger and Beuchat 2000). Testing seeds for contamination is problematic due to the low levels of contamination and nonuniform distribution of pathogens (Splittstoesser and others 1983).

The FDA has recommended testing spent irrigation water from sprout production for the presence of *E. coli* 0157:H7 and *Salmonella* spp. (FDA 1999). Testing spent irrigation water has many advantages over testing sprouts. In order to test the sprouts, multiple samples must be taken from various areas of the sprouting drum to ensure that the sampling is representative of the microflora present. Also, pummeling of the sprouts to break them open prior to testing, as recommended by the FDA (FDA 1999), may release phytoalexins inhibitory to the growth of some pathogens during enrichment or isolation (Jaquette and others 1996). The only disadvantage of testing spent irrigation water is that the level of microorganisms recovered is generally 1 log lower

than the level in the sprouts and low levels of pathogens may be missed (Fu and others 2001). However, if the testing of spent irrigation water is conducted at 48 h after the commencement of sprouting, as recommended by the FDA, the level of pathogens present in the irrigation water will be at a maximum level (Fu and others 2001; Splittstoesser and others 1983; Stewart 2001a,b) and may be more readily detectable.

For testing irrigation water, the FDA (1999) has recommended using VIP EHEC (Biocontrol Systems, Bellview, WA) or Reveal *E. coli* 0157:H7 tests (Neogen Corp., Lansing, MI) for the detection of *E. coli* 0157:H7, and Assurance Gold *Salmonella* EIA or Visual Immunoprecipitate (VIP) assay for *Salmonella* (both from Biocontrol Systems, Inc., Bellview, WA) for the detection of *Salmonella* spp. However, the *E. coli* tests require an overnight incubation in modified buffered peptone water with three added antibiotics, and the *Salmonella* methodology requires preenrichment and enrichment for approximately 48–50 h before testing. Thus, there is a need to develop sensitive and specific alternatives that can be completed in shorter time periods.

Time-Resolved Fluorescence of Lanthanide Cations

The involvement of 4f orbitals in the electronic structure of lanthanide (La) cations such as europium permits a transfer of excitation energy from ligands to central La cations prior to the emission of ion fluorescence that is characteristic by a relatively long fluorescence half-life (~50–1000 msec) and a considerable Stoke's shift (>200 nm) between the absorption and emission maxima. In contrast, the fluorescence half-life and Stoke's shift of common organic and biochemical compounds are in the range of 1–1000 μsec and 20–100 nm, respectively. Thus, with a pulsed excitation, the fluorescence of La may be easily separated from the interference fluorescence and scattered excitation light by delaying the emission measurement (time-resolved fluorescence, TRF). In addition, the quantum yield of La-chelates is usually quite high, e.g., 0.18 for Eu-(4,4,4-trifluoro-[Z-thienyl-l,3-butanedionato]) at 614 nm (Halverson and others 1964). A combination of time-delayed fluorescence and the unique properties of La-chelates has led to the development of a new technique called *dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA)*. In this technique, antibodies are modified to contain binding groups capable of forming very low fluorescence La-complex. The modified antibodies are used to capture target species and the antibody-bound La cations are then extracted out by an "enhancement solution" that contains chelates capable of forming strongly fluorescent products (Tu and others 2001b).

Time-Resolved Fluorescence Approach (TRF)

We have applied TRF measurement in combination with immunomagnetic capture to develop a sensitive and rapid method for pathogen detection (IMB-TRF). This approach has been demonstrated to detect *E. coli* and *Salmonella* in ground meats at a 1 CFU/g level and to show that nontarget microorganisms do not interfere with the detection (Tu and others 2002). In the experiment, europium (Eu) or samarium (Sm) labeled antibodies to the bacteria were incubated with IMB captured target organisms. Excess labeled antibody was washed away and the remaining beads were eluted into an "enhancement buffer," which contained chelators that extracted Eu or Sm to form strongly fluorescent products. The samples were then read in a Victor 1420 Multilabel

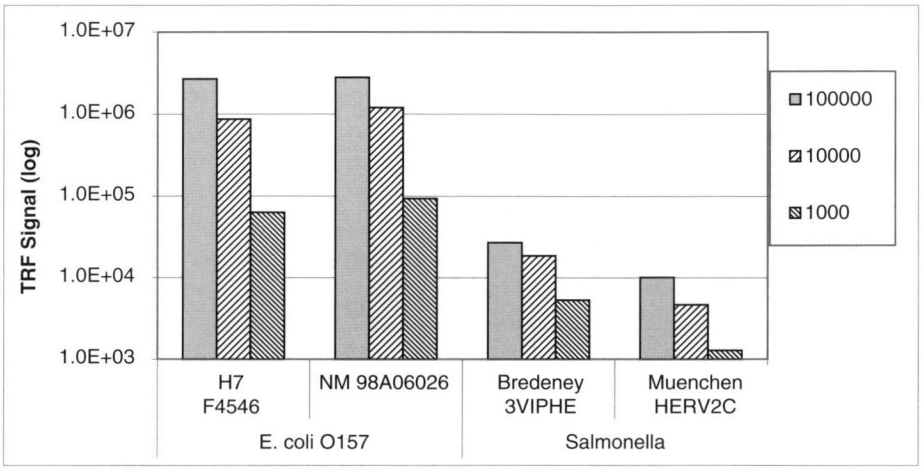

Figure 18.6. The sensitivity of IMB-TRF method for detecting different strains of *E. coli* and *Salmonella*.

Counter that provided pulsed excitation and delayed emission measurements (PerkinElmer Wallac, Turku, Finland).

Figure 18.6 shows the sensitivity of the IMB-TRF method for detecting different strains of *E. coli* and *Salmonella*.

Bacterial samples with indicated concentrations were first treated with proper IMBs. The captured bacteria were then reacted with either Eu-labeled (for *E. coli*) or Sm-labeled (for *Salmonella*) antibodies prior to TRF measurements (Tu and others 2002). As shown, detection limits were thus 750 CFU/ml for *E. coli* and 250 CFU/ml for *Salmonella*. Similar trends were found with *Salmonella* strains Stanley HO558, Anatum 4317, Infantis F4319, and Newport H1275 (data not shown).

To test the feasibility of using the developed IMB-TRF method to detect the pathogens in the alfalfa sprouts, experiments shown in Figure 18.7 were performed (Tu and others 2003b). Commercially obtained alfalfa sprouts were inoculated with the pathogens at indicated levels and then "stomachered" and incubated for 4.5 h at 37 °C. Data shown indicated that with crushed sprouts, the IMB-TRF could easily detect the presence of *E. coli* O157:H7 and *E. coli* O157:NM. However, the approach failed to detect *Salmonella* under the experimental conditions. The basis for this has yet to be determined but it has been reported by Castro-Rosas and Escartin (2000) that *Vibrio cholerae* O1 and *Salmonella typhi* showed no growth when inoculated onto alfalfa sprouts 24 h after germination. They attributed this observation to the abundance of competing background microflora at 24 h into the germination process.

Detection of Pathogens in Laboratory-Cultivated Sprouts Grown from Inoculated Seeds

Results of Figure 18.6 indicated that using whole alfalfa sprouts for pathogen detection by IMB-TRF might have its drawbacks. Thus, we decided to germinate contaminated

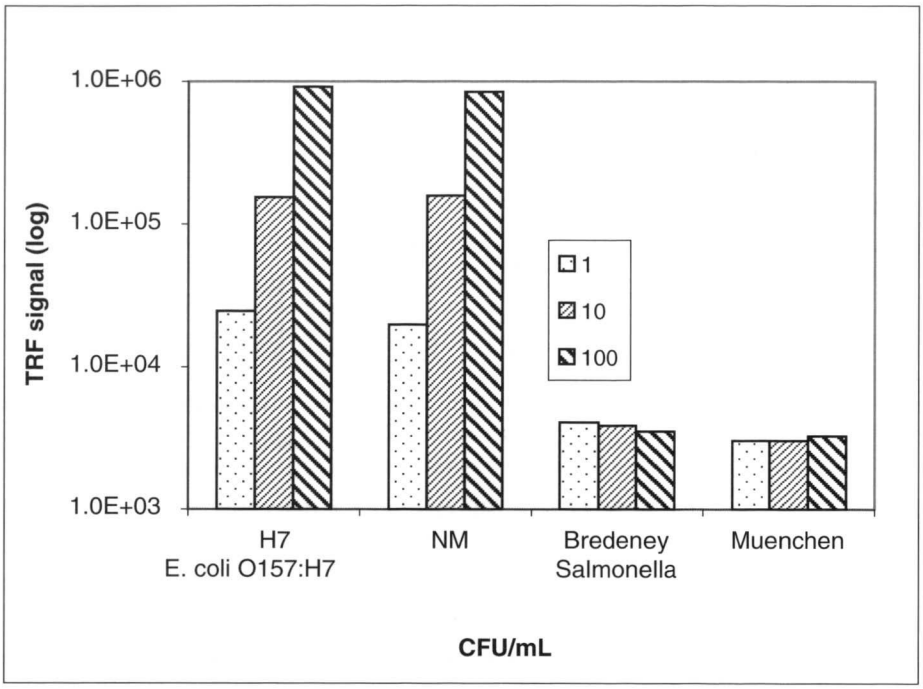

Figure 18.7. Detection of pathogens on crushed sprouts.

alfalfa seeds (4 CFU/g) in our laboratory using sterile Mason jars and sterile tap water for irrigation. Thus, an analysis of spent irrigation water should indicate whether the sprouts, and therefore the seeds, are contaminated by pathogens. Experimentally, alfalfa seeds artificially contaminated with *E. coli* O157 or *Salmonella* were used to produce sprouts (Tu and others 2002). As shown in Figure 18.8, water sample analyses were applicable for the detection of both *E. coli* O157 and *Salmonella*.

Detection of Pathogens in Irrigation Water and Sprouts

Although spent irrigation water testing is recommended by the FDA (1999), it may give false negative results because microbial counts in the irrigation water are, on the average, 1 log lower than those detected in sprout samples (FDA 1999; Fu and others 2001). For this reason, we chose to apply the developed detection method to both the water and sprouts simultaneously. We did not utilize the Seward Stomacher for pummeling sprouts germinated from lab-inoculated seeds because of complications described in Figure 18.6. Instead, whole sprouts were aseptically transferred to the proper culture medium for the enrichment. With this experimental design, both the sprouts and the spent irrigation water equally showed the presence of the pathogens as depicted in Figure 18.9. Unlike the results described in Figure 18.7, the use of sprouts germinated from contaminated seeds, under applied laboratory conditions, showed positive detection for both *E. coli* O157 and *Salmonella*. Apparently, the

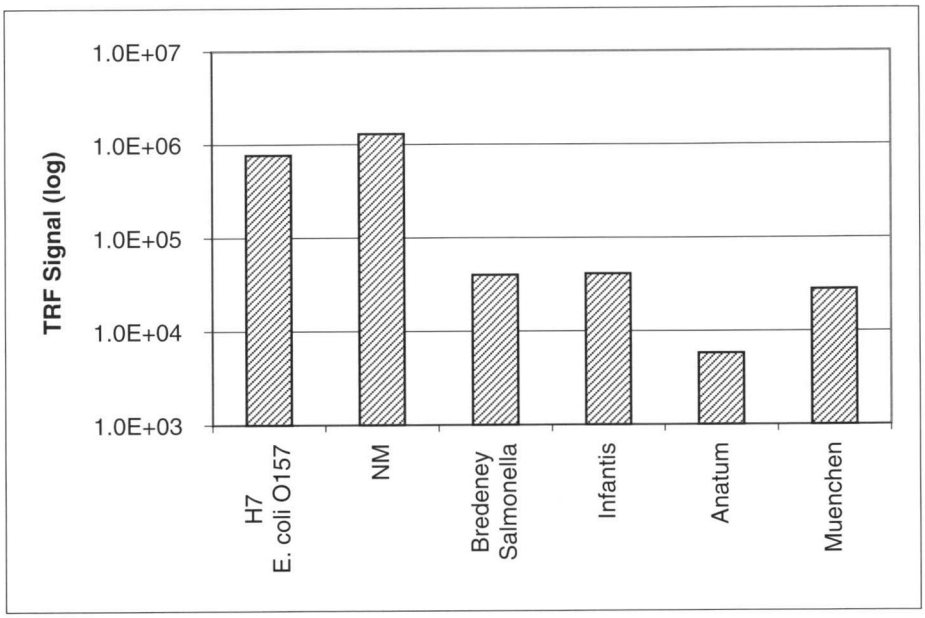

Figure 18.8. Detection of pathogens in irrigation water.

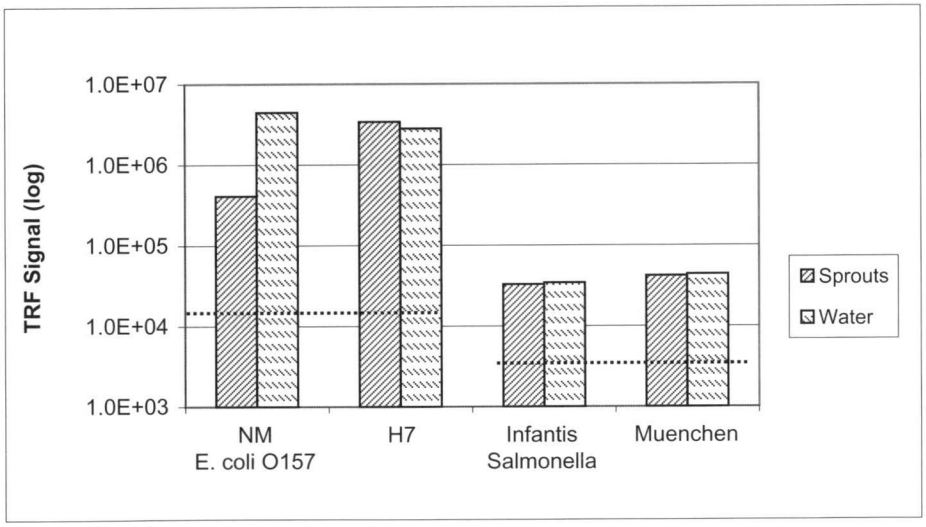

Figure 18.9. Detection of pathogens in sprouts germinated from contaminated seeds.

competitive exclusion of pathogens by background microflora is minimized by the use of pathogen-inoculated seeds, and this methodology more closely approximates naturally occurring contamination of seeds.

Conclusions

The number of outbreaks linked to fresh produce reported to the CDC has increased in the last years (Bean and Griffin 1990; CDC 2000). This increase may be due in part to improved surveillance, but other factors may also come into play. Proposed reasons include the significant increase in the consumption of fresh produce in the United States due to the growing awareness of fruits and vegetables as a part of a healthy diet. Also, greater volumes of minimally processed produce are being shipped from central locations and distributed over much larger geographical areas to meet the increased popularity of salad bars. This, coupled with increased global trade, significantly increases human exposure to a wide variety of foodborne pathogens and also increases the chances of outbreaks (Harris and others 2003).

To minimize the possibility of outbreaks, producers such as the International Sprout Growers Association (ISGA) have taken positive steps to address this problem by pursuing the use of 2% calcium hypochlorite for soaking alfalfa seeds prior to germination and growth. This intervention method has the potential to substantially reduce, but not necessarily eliminate, pathogenic microbial contamination of seeds that can be passed on to the consumer through ingestion of raw sprouts. Thus, the development of effective technologies that can be applied to detect pathogenic bacteria in produce is desirable.

In this chapter, we provide evidence demonstrating that a combination of IMB to capture and biosensors to detect (a method that was originally developed for detecting pathogens in meats) has the potential to detect low levels of pathogenic bacteria in produce, specifically cantaloupes and alfalfa seeds and sprouts. The sensitivity of developed IMB-TRF processes allows rapid detection, within an 8-h shift, of select pathogens even in the presence of high levels of the background microflora. In principle, the technology may be applied to the detection of pathogens in other produce that are of outbreak concerns. The availability of magnetic bead manipulator and biosensor detector in 96-well formats will certainly increase the feasibility of high-throughput screening of pathogens in meats and produce.

Disclaimer

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture or any other federal agency or governmental entity over others of similar nature not mentioned.

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